筑波大学
University of Tsukuba

The 16th World Lake Conference
November 7-11th , 2016
Bali, Indonesia

Influence of Dissolved Oxygen Concentration Change on Musty Odor Production by Actinomycetes

Azusa Hashimoto[1], Kazuya Shimizu[2],
Norio Sugiura[3], Motoo Utsumi[3]

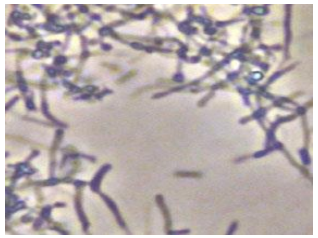
[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba*
[2] *Faculty of Life Sciences, Toyo University*
[3] *Faculty of Life and Environmental Sciences, University of Tsukuba*

Table of Contents

Motivation

Musty odor problem

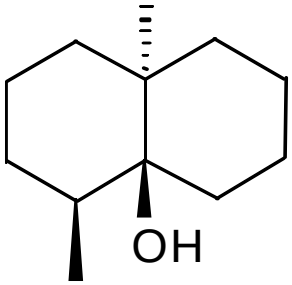
Microorganisms



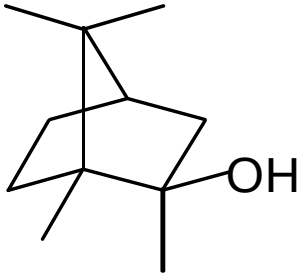
Actinomycetes

Cyanobacteria

Musty odor compounds

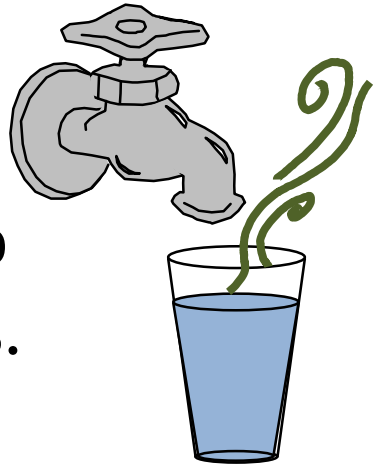


Geosmin



MIB

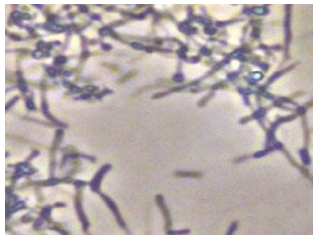
Unpleasant odor in drinking water leads to consumer complaints and economic losses.



Motivation

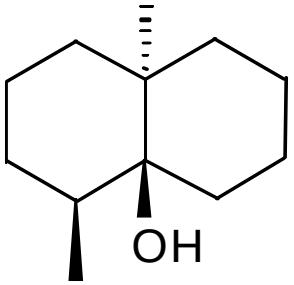
Influence of environmental factors

Microorganisms

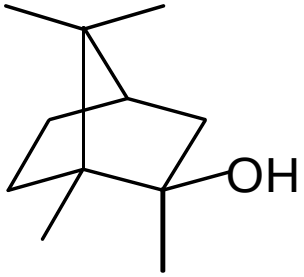


Actinomycetes Cyanobacteria

Musty odor compounds



Geosmin



MIB

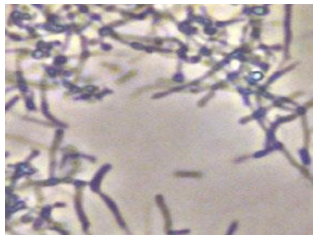
As for geosmin production by actinomycetes…

- pH and temperature (Sugiura, 1989)
- Carbon sources, phosphorus concentration, and several metals (Schrander and Blevins, 2001)

Motivation

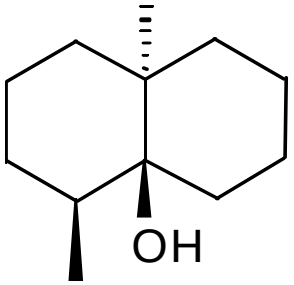
Influence of environmental factors

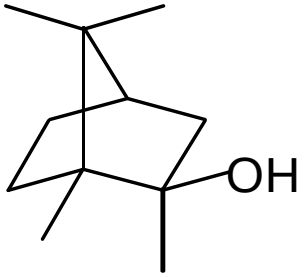
Microorganisms



Actinomycetes Cyanobacteria

Musty odor compounds





Geosmin MIB

Environmental factors of sediment

?

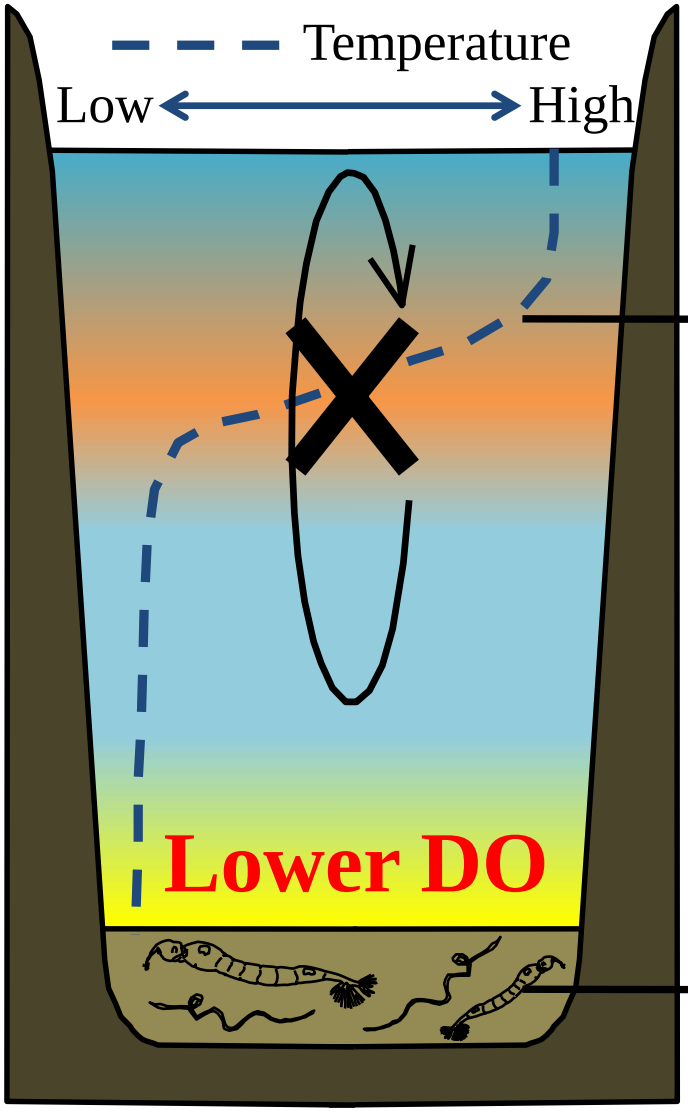
Growth and geosmin production by actinomycetes

As for geosmin production by actinomycetes:

- pH and temperature (Sugiura, 1989)
- Carbon sources, phosphorus concentration, and several metals (Schrader and Blevins, 2001)

Motivation

Dissolved oxygen (DO) concentration change



Thermocline is developed.

↓

Vertical water mixing stops.

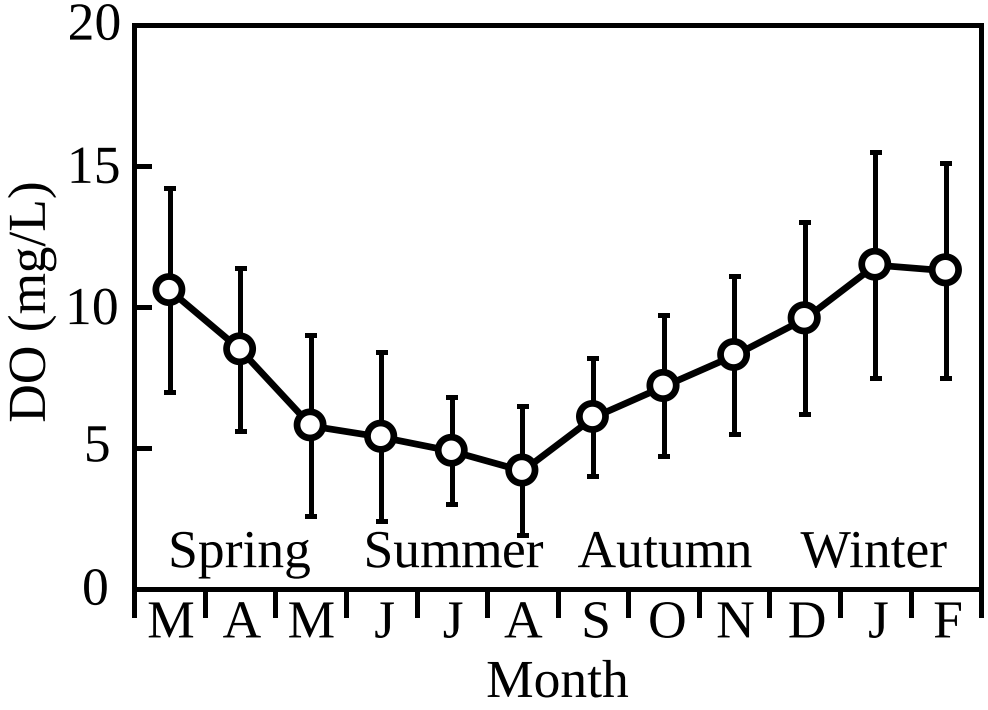
↓

Oxygen supply
to bottom layer stops.

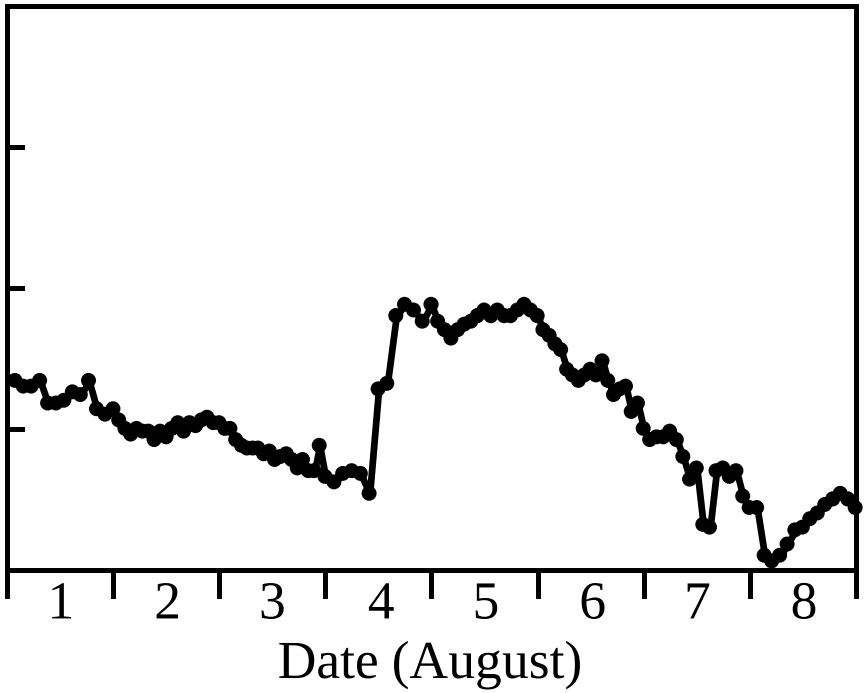
Oxygen is consumed by
degradation of organic matter.

Motivation

Dissolved oxygen (DO) concentration change



(Lake Kasumigaura database 2001-2012)



(Ishikawa *et al.*, 1989)

Fig. DO concentration above sediment in Lake Kasumigaura, Ibaraki, Japan.

It has not been investigated
whether or not changing DO concentration affect
geosmin production by actinomycetes.

Objective

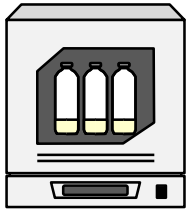
Evaluate the effect of different DO concentration on growth and geosmin production by *Streptomyces coelicolor* A3(2)

Preculture

DO 8 mg/L

Dark condition,
30ºC,
120 rpm,
2 days

Culture at different DO concentration

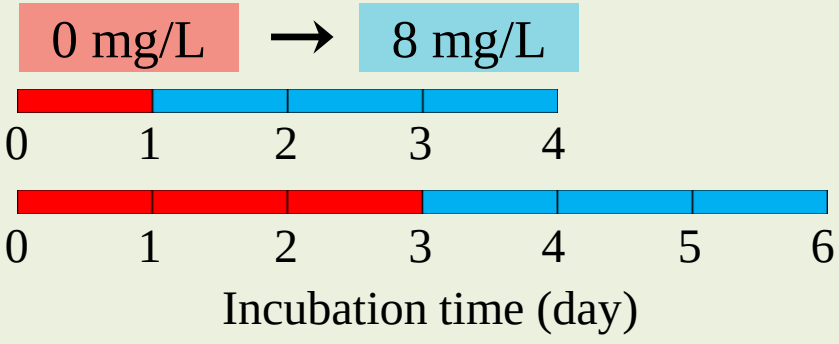


Dark condition,
30ºC,
120 rpm

Initial DO concentration

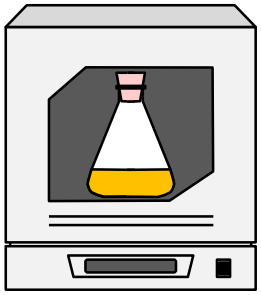
0, 0.4, 2, 4, 8 mg/L

Temporally changing DO concentration



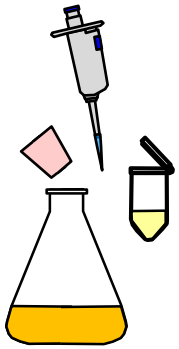
Materials and Methods

Preculture under aerobic condition (DO 8 mg/L)



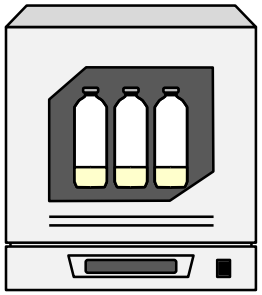
30ºC, 120 rpm, 2 days

Harvest and wash



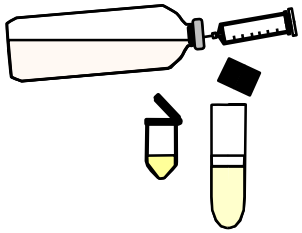
Inoculation into new media

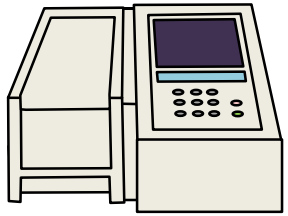
Incubation

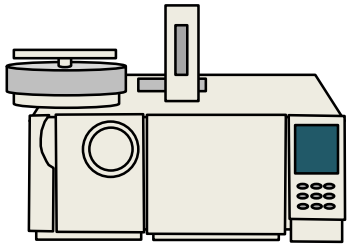


30ºC, 120 rpm, 3 days

Measurement of geosmin concentration and optical density (OD_{450})





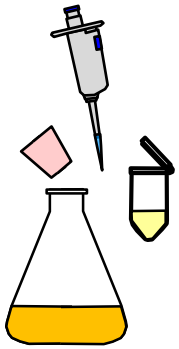


Materials and Methods

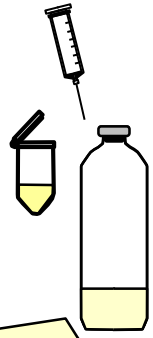
Preculture under aerobic condition (DO 8 mg/L)

30ºC, 120 rpm, 2 days

Harvest and wash

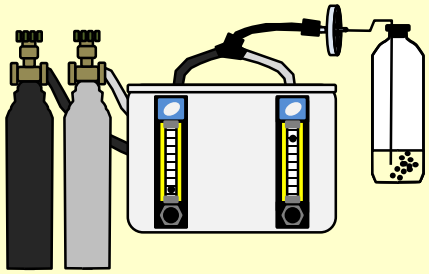


Inoculation into new media



Effect of initial DO concentration

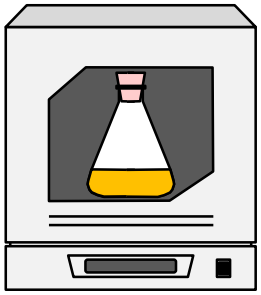
Purge with mixture of O_2 and N_2



DO (mg/L)	Purge with
0	100% N_2
0.4	99% N_2 + 1% O_2
2	95% N_2 + 5% O_2
4	90% N_2 + 10% O_2
8	Air

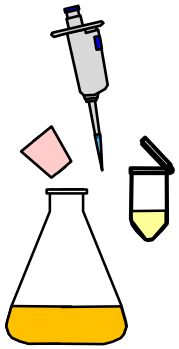
Materials and Methods

Preculture under aerobic condition (DO 8 mg/L)



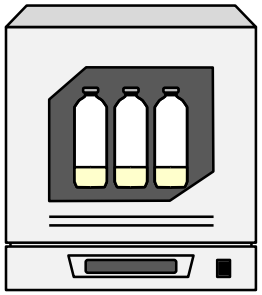
30ºC, 120 rpm, 2 days

Harvest and wash



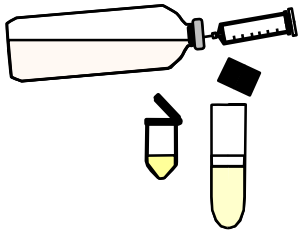
Inoculation into new media

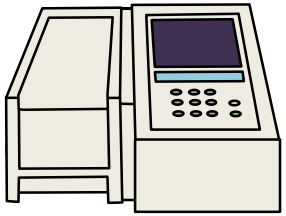
Incubation

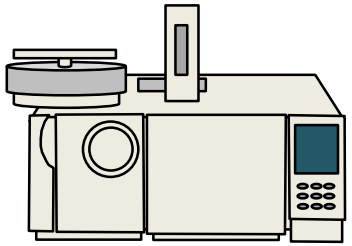


30ºC, 120 rpm, 3 days

Measurement of geosmin concentration and optical density (OD_{450})







Materials and Methods

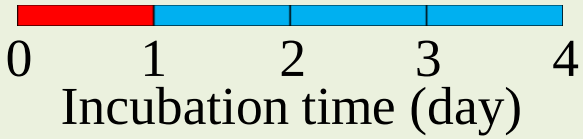
Preculture under aerobic condition (DO 8 mg/L)

Harvest and wash

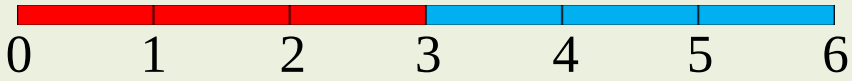
Inoculation into new media

Effect of temporally changing DO concentration

- Anaerobic condition (0 mg/L) for 1 day,
  and then aerobic condition (8 mg/L) for another 3 days

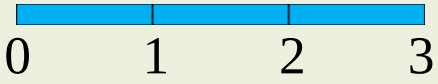


- Anaerobic condition (0 mg/L) for 3 days,
  and then aerobic condition (8 mg/L) for another 3 days



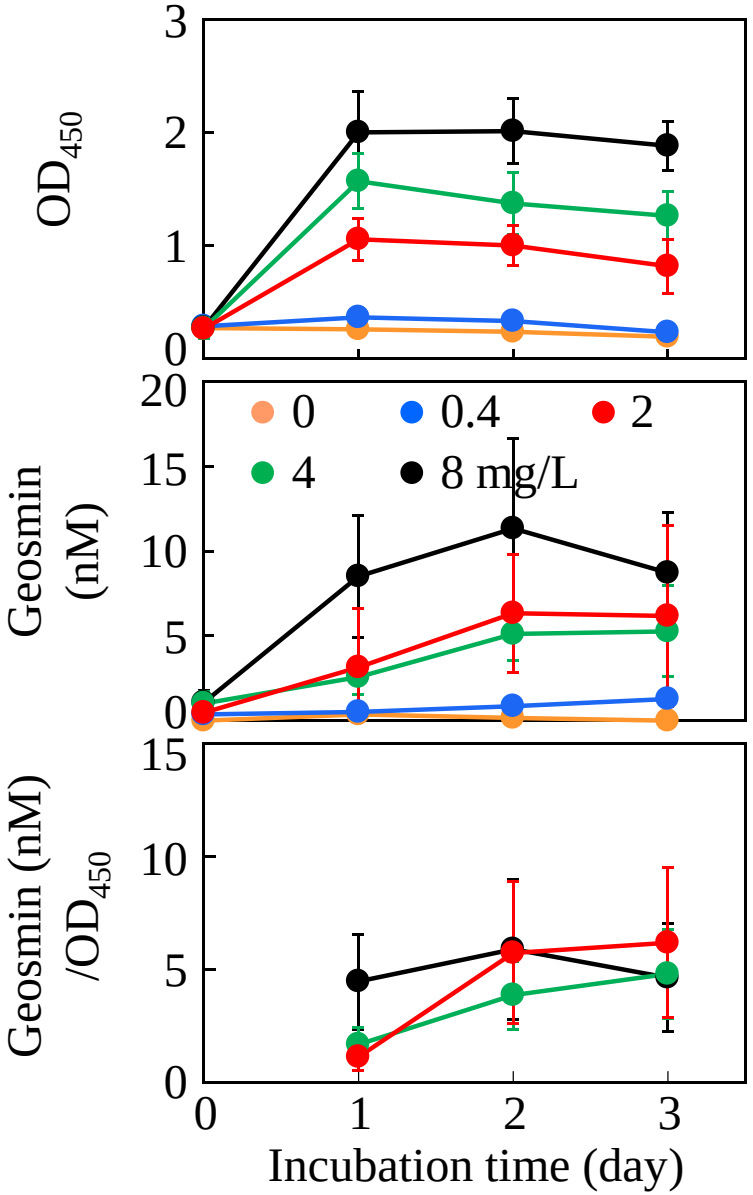
- Anaerobic control (0 mg/L)

- Aerobic control (8 mg/L)



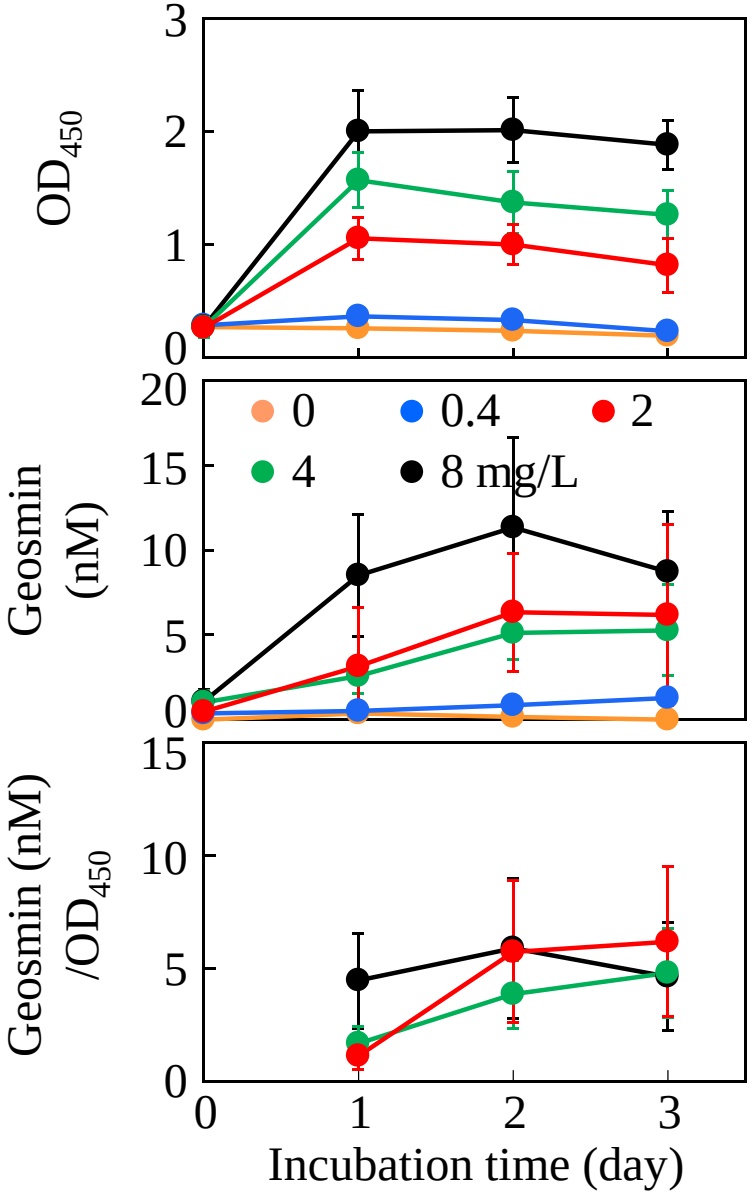
Results and Discussion

Effect of initial DO concentration



Results and Discussion

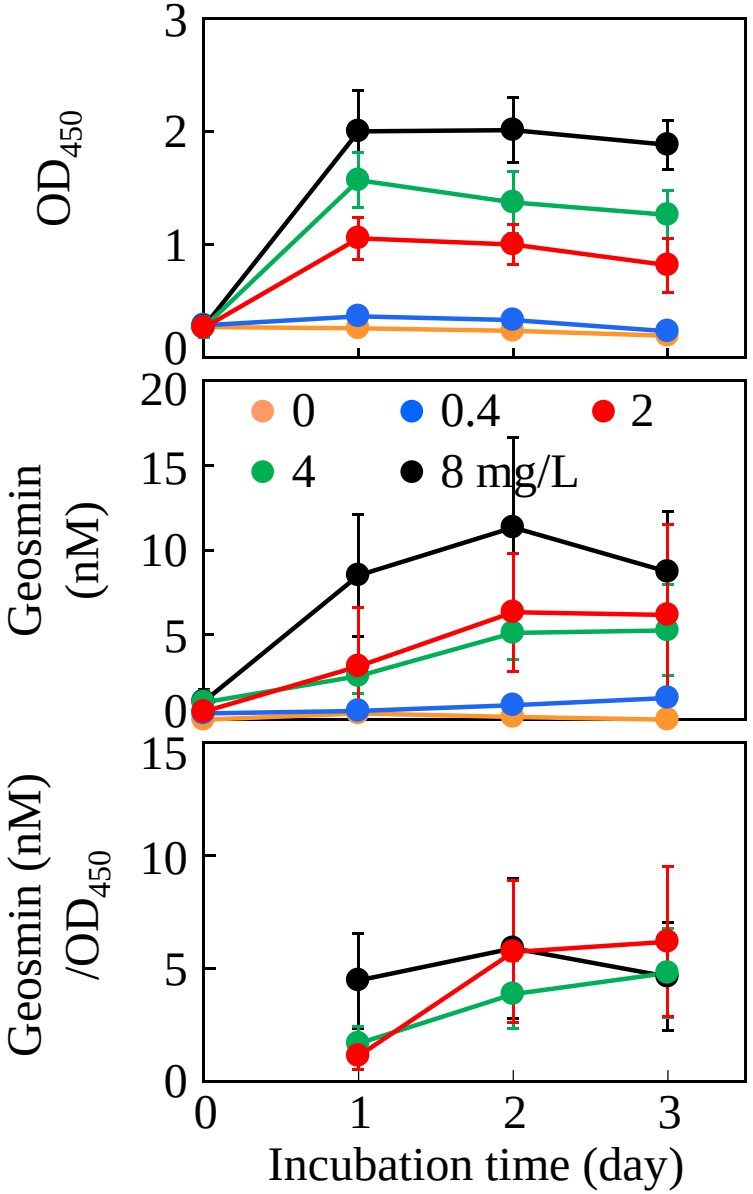
Effect of initial DO concentration



- Growth was not observed (● , ●).
- Logarithmic phase was observed from day 0 to day 1, and stationary phase was observed from day 1 to day 3 (●, ●, ●).
- The ratio of OD_{450} (2 and 4 mg/L) to 8 mg/L condition were 50% (●) 80% (●) on day 1, respectively.

Results and Discussion

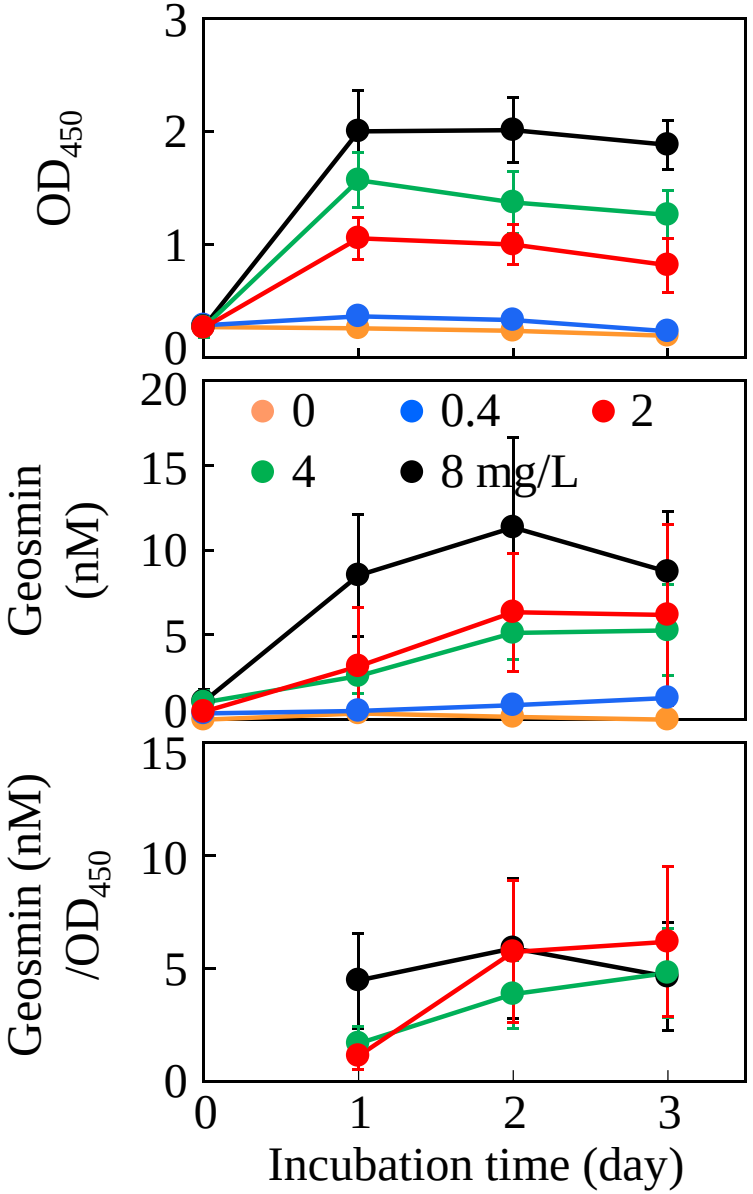
Effect of initial DO concentration



- Geosmin was not produced (●,●).
- Geosmin was produced during logarithmic and early stationary phase (●, ●, ●).

Results and Discussion

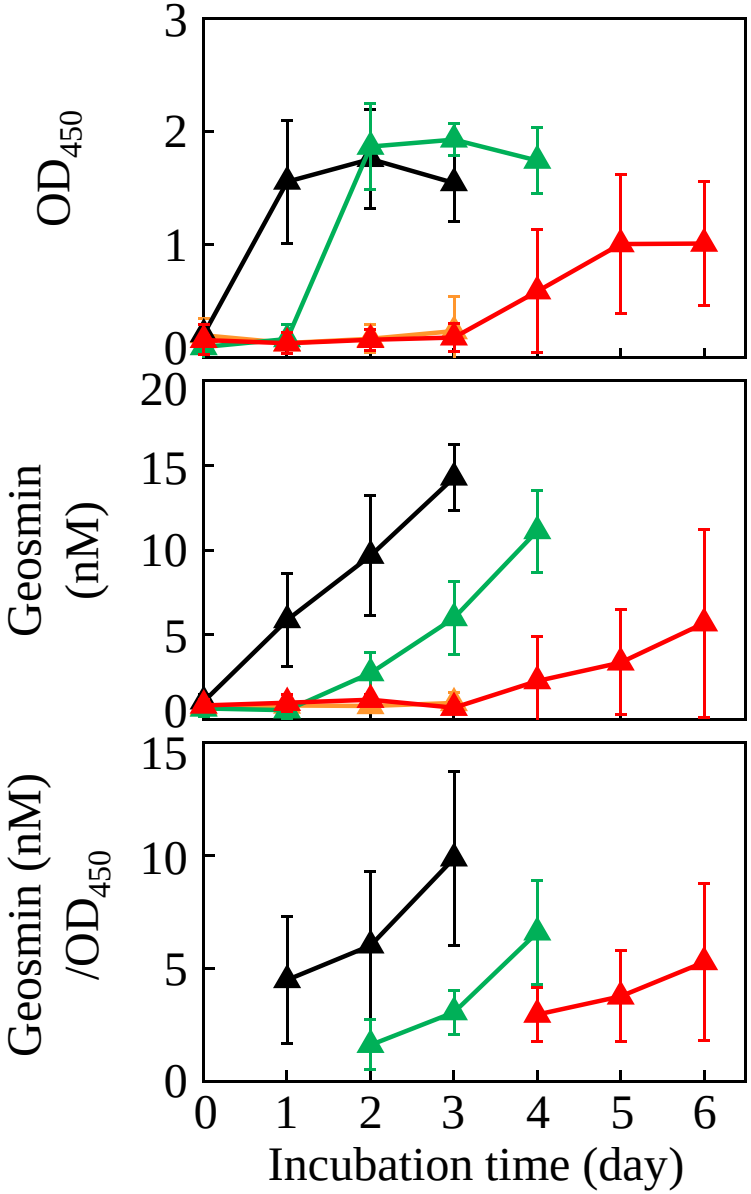
Effect of initial DO concentration



- Higher DO concentration showed higher geosmin production activity of each cell during logarithmic phase (●).
- There were not significant differences in geosmin production activities of each cell during stationary phase (●, ●, ●).

Results and Discussion

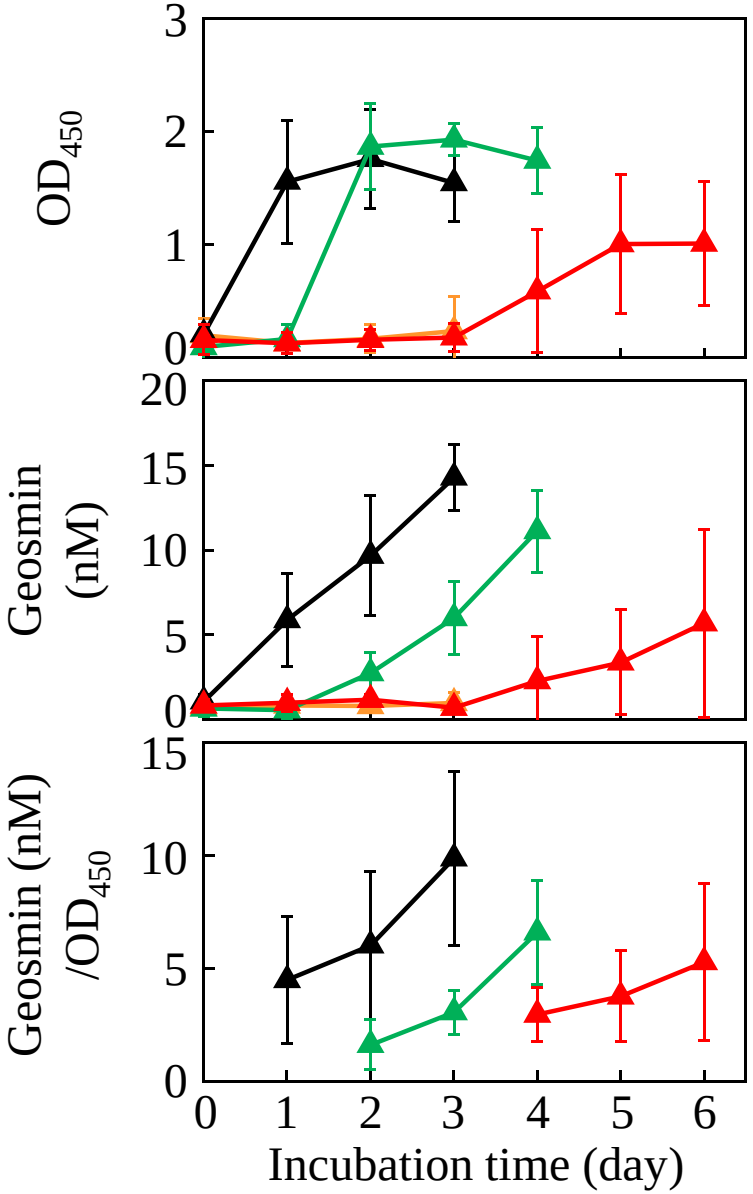
Effect of temporally changing DO concentration



- 1 day anaerobic, 3 days aerobic condition
- 3 days anaerobic, 3 days aerobic condition
- Aerobic control (8 mg/L)
- Anaerobic control (0 mg/L)

Results and Discussion

Effect of temporally changing DO concentration



- Similar growth was observed (▲, ▲).
- Logarithmic phase lengthened into 2 days (▲).
- The ratio of OD_{450} to aerobic control or 1 day anaerobic, 3 days aerobic condition was 60% during stationary phase (▲).

▲ 1 day anaerobic, 3 days aerobic condition

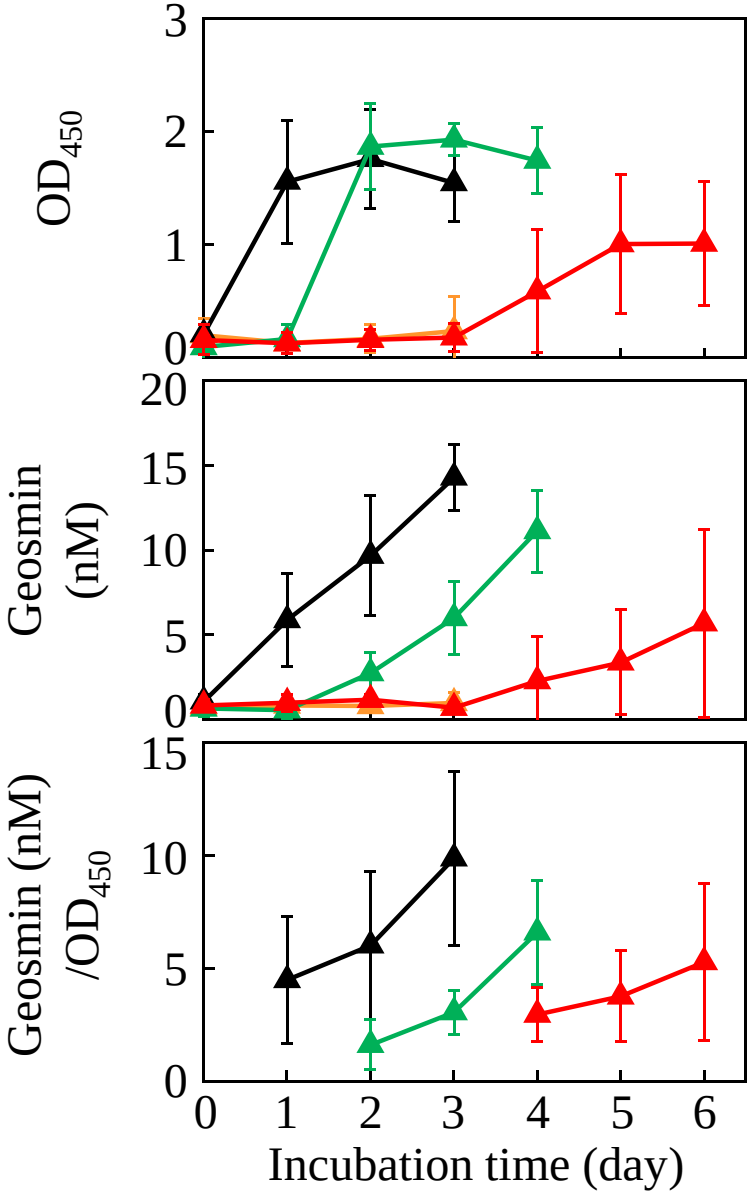
▲ 3 days anaerobic, 3 days aerobic condition

▲ Aerobic control (8 mg/L)

▲ Anaerobic control (0 mg/L)

Results and Discussion

Effect of temporally changing DO concentration



- Final geosmin amount was lower than aerobic control (▲, ▲).
- Final geosmin amount was much lower than 1 day anaerobic, 3 days aerobic condition (▲).

▲ 1 day anaerobic, 3 days aerobic condition

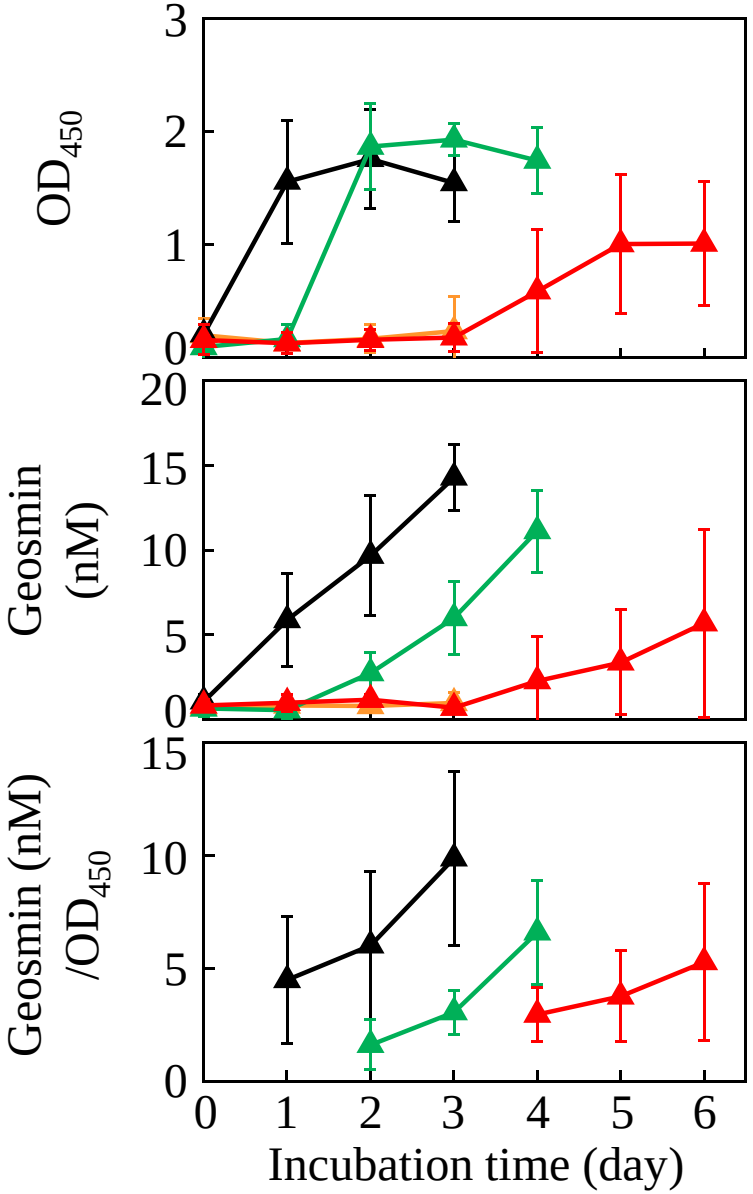
▲ 3 days anaerobic, 3 days aerobic condition

▲ Aerobic control (8 mg/L)

▲ Anaerobic control (0 mg/L)

Results and Discussion

Effect of temporally changing DO concentration



- The ratios of geosmin production activities of each cell to aerobic control were 70% (▲) and 50% (▲) on the final day, respectively.
- The slope of ▲ was smaller than that of ▲ .

▲ 1 day anaerobic, 3 days aerobic condition

▲ 3 days anaerobic, 3 days aerobic condition

▲ Aerobic control (8 mg/L)

▲ Anaerobic control (0 mg/L)

Conclusions

Effect of initial DO concentration

- Initial DO concentration affected growth and geosmin production by *S. coelicolor* A3(2).
- Growth and geosmin production were not observed below 0.4 mg/L condition.

Effect of temporally changing DO concentration

- Prolonged exposure to anaerobic condition resulted in depression of growth and geosmin production.
- Slopes of growth and geosmin production decreased with longer anaerobic condition.

These results indicated that *in situ* DO condition should affect growth and geosmin production by actinomycetes.

Thank you for listening.